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Development and Validation of Green Stability-Indicating RP-HPLC Method for Estimation of Ertapenem: Degradation Kinetics and Structural Mass Elucidation Using MS

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Abstract

Ertapenem is an antibiotic that is used in the treatment of several bacterial infections, and it was recently approved for the treatment of the COVID-19 virus. A simple validated stability-indicating reversed phase High Performance Liquid Chromatographic (RP-HPLC) method was developed for ertapenem estimation. The separation was done using Pronto SIL® C18 (300 × 4.6 mm, 5 µm) as the stationary phase, methanol, and acidified water as the mobile phase in gradient mode with a flow rate of 1 mL/min and a pH 4.0 adjusted by orthophosphoric acid. The method was validated according to ICH guidelines. The linearity ranges from 5.00-120.00 µg/mL with LOD 1.60 µg/mL and LOQ 4.85 µg/mL. Afterwards, the degradation behaviour of ertapenem was studied under various stress conditions, including acidic, basic, oxidative, thermal, and photolytic. The degradation study showed stability of ertapenem under thermal, photolytic degradation and severe degradation under acid, basic and oxidative conditions. The kinetics parameters were studied for acidic and basic degradation. The parameters showed that it follows a pseudo-first order reaction. Degradation products were structurally elucidated using mass spectrometry. The method has been evaluated as an excellent green analytical method based on eco-scale penalty points and the analytical greenness metric approach.

Keywords: Ertapenem; Forced degradation; HPLC; Degradation kinetics; Mass Spectrometry, COVID-19

1. Introduction

Ertapenem (ERTA) **(Fig. 1)**, is a carbapenem antibiotic that is utilized in curing many bacterial infections, including gram positive, gram negative and aerobic bacteria. It is effective in the treatment of patients with abdominal infections, urinary tract infections, and community-acquired pneumonia [1]. Moreover, recently, in the COVID-19 pandemic, ERTA proved its efficacy in urinary tract infections treatment and reduced hospital admission rates as it decreased use of parenteral antimicrobial therapy that requires hospitalization [2,3].It belongs to beta-lactam antibiotics like cephalosporins and penicillin. Its mechanism of action depends on inhibition of synthesis of bacterial cell wall by attaching to penicillin-binding protein and interfering with peptidoglycan formation [4]. The plasma protein-binding drug of ERTA is about 94% so it has a long half-life, which allows for a single daily dose [5]. A parenteral dose (1g) is administered to adults once daily. The drug is renally eliminated and not metabolized by the liver, which is why the dose is reduced in renal insufficiency diseases. Since ERTA does not interfere with p-glycoprotein and cytochrome P450-mediated drug clearance, drug-drug interactions are rare [5].

A literature review survey revealed that ERTA was analyzed using different analytical techniques, such as spectrophotometric methods [6–8], spectrofluorimetric methods [9,10]. chromatographic methods [11–15] and electrochemical methods [16–18].

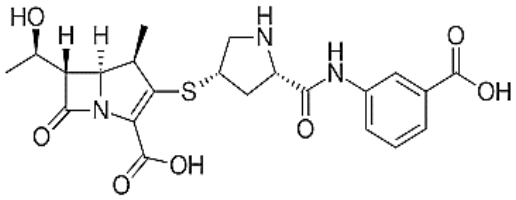

Figure 1. Chemical structure of ERTA.

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The chemical stability of the pharmaceutical compounds is an important issue because it affects the safety and effectiveness of medications. Moreover, stability studies are crucial for patient health as an unstable product will not only harm the patient with side effects but also affect the pharmacological activity of the drug; it may decrease the efficacy of the drug up to 85% of its claimed concentration [19]. Thus, before approval of a pharmaceutical product, stability data should be provided.
Also, stability data aids in choosing the best formulation, packaging, and best storage conditions. Thus, to evaluate drug stability, forced degradation studies are performed, in which the drug is degraded under extreme conditions producing degradants that are analyzed to assess its stability [20].
One of the most used techniques in pharmaceutical analysis is HPLC. It is frequently utilized in the quality control of pharmaceutical formulations, assessing the stability of formulations under specified time, identifying degradation products, characterizing impurities, and drug analysis in biological matrices [21–23].
Chemical reagents have the potential to cause harm to the environment and the health of humans. Thus, evaluation of greenness of the analytical methods are crucial for promoting environmental sustainability, reducing waste generation, conserving resources, ensuring occupational safety, complying with environmental regulations, realizing cost savings, and demonstrating social responsibility [24–27].
Up to our knowledge and according to the literature survey, there are very few methods for evaluating the stability of ERTA under different conditions including spectrophotometry [28,29], chromatography [30–34], there are no reported degradation kinetics studies for the obtained degradation products and only one method that was evaluated by greenness assessment tools. Therefore, the aim of this research is to develop a simple validated HPLC method for quantification of ERTA. Study the degradation behavior of ERTA under different stress conditions including acidic, basic, oxidative, photolytic and thermal. For the first time, calculation of kinetic parameters for the obtained degradants. Structural elucidation of the obtained degradation products using mass spectrometry technique. Finally, Evaluation of eco-friendless of the method using two greenness assessment tools including analytical-eco scale and AGREE software.

2. Experimental

2.1. Materials and chemicals

ERTA pure powder with certified purity of 99.90% was purchased from Sigma Aldrich pharmaceutical company (Dermstadt, Germany).
Methanol and Orthophosphoric acid were purchased from Fisher Scientific (Loughborough, UK).
Hydrochloric acid (HCl) and sodium hydroxide (NaOH) was purchased from (Adwic, Cairo, Egypt).

Hydrogen peroxide was provided from (Adwic, Cairo, Egypt).

2. Instruments

UHPLC 3000 (Germering, Germany): pump (ISO-3100SD), autosampler (WPS-3000SL), column thermostat (TCC-3000SD) and photodiode array detector (PDA-3000RS) (Germering, Germany).
Stationary phase: Pronto SIL® C18 (300 × 4.6 mm, 5 µm particle).
Water purification: MILIQ water Thermo scientific (Hungary, Europe).
pH meter: Jenway pH-meter 3310 (Essex, United Kingdom).
Thermostatic water bath: (Wisd-WSB-18, Berlin, Germany).
Oven: Binder (Germany).
UV lamp: 6 W power (Upland, USA).
Triple quadrupole mass spectrometer: Agilent (California, USA).

2.3. Procedures

2.3.1 Preparation of standard solution

ERTA standard solution was prepared by dissolving 10.00 mg of pure powder in deionized water in a 10 mL volumetric flask to obtain a final concentration 1.00 mg/mL and the volume was completed with deionized water.

2.3.2. Optimum Chromatographic Conditions

Chromatographic separation was done using Pronto SIL® HPLC C18 column (300 × 4.6 mm, 5 µm). The mobile phase is composed of methanol and acidified water in gradient mode as illustrated in **Table. 1**. pH was adjusted at 4.0. The flow rate was 1.0 mL/min. Wavelength was maintained at 224.0 nm. The column oven was adjusted at 30ºC. Injection volume was 10 µL.

Table. 1 Gradient mode for ERTA separation.

Time (min)	Mobile phase (A) (%)	Mobile phase (B) (%)
0	10	90
15	90	10
16	10	90
21	10	90

2.3.3 Calibration curve construction

A standard solution of ERTA (1.00 mg/mL) was used to prepare different solutions. In a series of 10-mL volumetric flasks, 0.05, 0.10, 0.30, 0.50, 0.70, 1.00 and 1.20 mL were transferred from the standard solution and diluted with deionized water to obtain concentrations of 5.00, 10.00, 30.00, 50.00, 70.00, 100.00, and 120.00 µg/mL. the prepared solutions were injected three times into the HPLC instrument under previously optimum conditions.

2.3.4 Forced degradation studies

Stability testing was performed according to the International Committee for Harmonization (ICH) Q1A (R2) [35].

2.3.4.1 Acid induced hydrolysis

For acidic hydrolysis, 2N HCl was prepared by dissolving 4.89 mL of HCl in deionized water in a 100- mL volumetric flask. Then 5.0 mL of 2N HCl with 1.0 mL of drug (1.00 mg/mL) was transferred into a 10.0 mL volumetric flask and the volume was completed with deionized water to obtain a concentration of 100.00 µg/mL. The solutions were left on the water bath at 65 ºC and after interval time 5, 15, 30, 60, 120 min the samples were neutralized with sodium hydroxide to pH 7.00. The prepared solutions were injected into HPLC system in triplicates. Afterwards, the degradation kinetics was studied after 5, 15, 30, 60, 120 min.

2.3.4.2 Basic induced hydrolysis

For basic hydrolysis 2N NaOH was prepared by dissolving 8.00 g of NaOH in deionized water in a 100-mL volumetric flask. Then, 5 mL of 2N NaOH with 1.0 mL of drug (1.00 mg/mL) was transferred into a 10-mL volumetric flask, and the volume was completed with deionized water to obtain a concentration of 100.00 µg/mL. The solutions were left on the water bath at 65 ºC. After interval time 5, 15, 30, 60, 120 min; the samples were neutralized with HCl till pH 7.00. Then, degradation kinetics was studied after 5, 15, 30, 60, 120 min.. Afterwards, the previously prepared solution was injected into HPLC system in triplicate.

2.3.4.3 Oxidative hydrolysis

In a 10-mL volumetric flask, 1.0 mL of ERTA standard was added to 4 mL of 70% (v/v) hydrogen peroxide, and then the volume was completed with deionized water to obtain a concentration of 100.00 µg/mL. After time interval 5, 15, 30, 60, 120 min the reaction was stopped by a few drops of water. The solution was injected into HPLC system three times.

2.3.4.4 Photolytic degradation

Photolytic degradation was performed using a UV lamp and sunlight. ERTA powder (10.00 mg) was weighed accurately and exposed to a UV lamp for 6 hours. Then, the powder was transferred to 10-mL volumetric flask and the rest of volume was completed with deionized water to obtain concentration 100.00 µg /mL. After that, the prepared concentration was injected in triplicate in the HPLC system. Another 10.00 mg of ERTA powder was left in sunlight for 6 hours. The drug was transferred to 100-mL volumetric flask and the volume was completed with deionized water to obtain concentration of 100.00 µg/mL. The solution was injected three times into the HPLC instrument.

2.3.4.5 Thermal degradation

An accurately weighted 10.00 mg of ERTA powder was left in the oven for 24 hours at a temperature of 60ºC. The powder was transferred to a 10-mL volumetric flask and the volume was completed with deionized water. Then, a working solution of 100.00 µg/mL was prepared and injected three times into the HPLC system.

2.3.5. Mass spectrometry characterization

Structure elucidation was performed to determine the degradation pathway of the drug using MS. Samples of acid, base, and oxidative degradation was collected after 2 hours. Mass analysis was performed using a triple quadrupole mass spectrometer in negative mode. The temperature source was 100 ºC, while the temperature of desolvation was 300 ºC and ion spray voltage was 4000 v.

2.3.6. Analytical method Validation

The chromatographic conditions mentioned previously was validated according to ICH guidelines in terms of linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness [36].

2.3.6.1 System suitability

System suitability parameters were evaluated to make sure that the method was suitable for its intended use. Several parameters were tested, including retention time, tailing factor, capacity factor, number of theoretical plates, height equivalent to theoretical plates and resolution between the drug and degradation product.

2.3.6.2 Linearity

Linearity means the ability of the analytical technique to obtain results that are directly proportional to the concentration. Method linearity was assessed by preparing solutions having the concentration of5.00, 10.00, 30.00, 50.00, 70.00, 100.00, and 120.00 µg/mL from ERTA standard solution,1.00 mg/mL. Under the previously mentioned chromatographic conditions, 10.0 µL of each solution was injected three times. The calibration curve was constructed by plotting concentration against peak area average, and the regression equation was obtained.

2.3.6.3 Accuracy and precision

Accuracy refers to the closeness of the measured values to the true value. Three concentrations (10.00, 50.00, 100.00 µg/mL) were prepared; each concentration was injected three times. It was evaluated by percentage recovery.

$$\%Recovery = \frac{Actual\ concentration}{Theortical\ concentration} \times 100$$

Three different concentrations of ERTA (10.00, 50.00, 100.00 µg/mL) were used to assess intraday and inter-day precision. Intra-day precision was evaluated by injecting the three concentrations on the same day, while inter-day precision was evaluated by injecting the three concentrations on different days. Both intra-day and inter-day precision were evaluated by %RSD.

2.3.6.4 Specificity

Method specificity is evaluated by its ability to distinguish between drug peaks and degradation products peaks. Appearance of additional peaks in the chromatogram was checked.

2.3.6.5. Limit of detection and limit of quantification

LOD means the lowest concentration that can be detected. It was computed by the following rule:

$$LOD = \frac{3.3\ \sigma}{Slope}$$

LOQ means the lowest concentration that can be determined with accuracy and precision. It was computed by the following rule:

$$LOQ = \frac{10\ \sigma}{Slope}$$

Where, σ is the standard deviation of regression residuals

2.3.6.6. Robustness

Robustness means that the method remains unaffected by deliberate changes in chromatographic conditions. The temperature was changed ± 1 ºC, pH ± 1, wavelength ± 1 nm and flow rate ± 1 mL/min. Obtained results were explained by % RSD.

3. Results and discussion

Stability studies are very important as they show the changes that happen to the drug throughout their shelf life under the effect of different environmental conditions. Drug stability has an impact on product safety and effectiveness. Moreover, impurities resulting from degradation of the drug may result in a loss of efficacy as well as potential side effects. Thus, ensuring the chemical and physical stability of medications is crucial to guaranteeing their efficacy and safety.

ERTA is made up of a side chain of thiaproline amide and a highly strained beta-lactamase ring. Carbapenems strained ring structure led to their instability with varying pH levels [29]. This extremely strained ring structure hydrolyzes and forms an open-ring degradation product. Hence, it is crucial to perform stability or degradation studies to assess product degradation kinetics characteristics, potential side effects, and its availability in the market [37]. Therefore, a simple green-validated HPLC method was developed for ERTA separation from its degradation products produced under different stress conditions. Moreover, degradation kinetics behavior was studied. The degradants were characterized using mass spectrometry. The greenness of the method was evaluated to ensure that it was an environmentally friendly method.

3-1 Chromatographic conditions optimization

The effect of various chromatographic conditions was investigated including stationary phase, mobile phase composition, flow rate, pH, and wavelength to achieve the best separation.

Two columns have been tried: BDS Hypersil® C18 (150 × 4.6 mm, 5 µm particle) and C18 (300 × 4.6 mm, 5 µm). The first mentioned column was not able to separate the drug from the degradation product. The column Pronto SIL® with dimensions (300 × 4.6 mm, 5 µm) was able to separate ERTA from degradation product with sharp and uniform peaks.

One of the crucial factors that greatly affects the separation is the composition of the mobile phase. Therefore, different mobile phases were tried, including methanol: acidified water &acetonitrile: acidified water. It was first attempted to use acetonitrile and water with a ratio (70:30, v:v) as the mobile phase, but ERTA peak was not consistent and tailed. Increasing the ratio of organic phase does not enhance peak sharpness as the drug is highly soluble in water. Then, methanol and acidified water were tried in gradient mode after trying isocratic mode which was not able to separate between the drug and products of degradation. It was found that the optimum mobile phase was methanol and acidified water with a pH adjusted to 4.0 by orthophosphoric acid.

The optimum flow rate was 1.0 mL/min. Decreasing flow rate less than 1.0 mL/min resulted in retention time increase without improvement of separation. Increasing the flow rate to more than 1.0 mL/min increased the pressure of the pump.

Different values of pH were investigated including pH (4.0, 5.0, 6.0, and 7.0). The optimum pH was 4.0 as the ERTA pKa for the acidic part 3.22 and for the basic part 9.03. The base line of the peaks was distorted when the pH value increased more than 4.0. It was found that the best detection wavelength 224.0 nm. The wavelength choice was based on absorption spectrum λ max obtained by PDA detector. The spectrum of ERTA is shown in (**Fig. 2**).

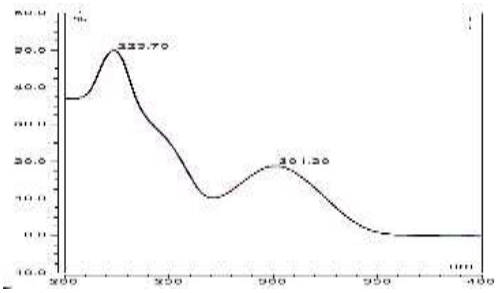

Figure 2. UV spectrum of ERTA extracted by HPLC chromatogram.

Under the previously mentioned conditions, ERTA was eluted at a retention time 7.48 min as illustrated in (**Fig 3**).

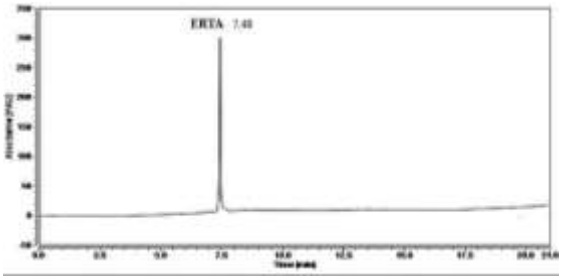

Figure 3. HPLC chromatogram of 100.00 µg/mL using C18 (300 × 4.6 mm, 5 µm particle) as stationary phase, methanol and acidified water as mobile phase with pH adjusted at 4.00, flow rate 1 mL/min, temperature maintained at 30ºC and UV detector at 224.0 nm.

3.2 Method validation

3.2.1. System suitability parameters

System suitability parameters were calculated and found to be in the specified range. The results of the calculated parameters and accepted criteria are shown in (Table 2).

Table. 2 System suitability parameters of suggested HPLC technique.

Parameter	ERTA	Reference value
Retention time (t_R) (min)	7.48	------
Tailing factor (T_f)	1.03	Less than 2
Capacity Factor (K)	2.21	>1
Number of theoretical plates (N)	3602	>2000
Height equivalent to the theoretical plates (HETP)	0.04	The smaller the value the higher the column efficiency
Resolution between drug and acidic degradant	5.26	≥1.50
Resolution between drug and basic degradant	15.26	

3.2.2. Linearity

Under optimized chromatographic conditions, the method was found to be linear from 5.00-120.00 µg/mL with a correlation coefficient 0.9997. Calibration curve is shown in (**Fig. 4**). R2 near 1 was the accepted criteria for acceptance of the linearity. Validation parameters are illustrated in **Table 3**.

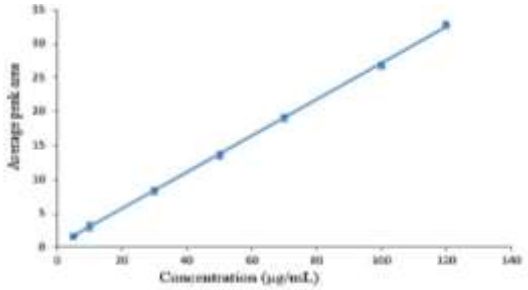

Figure 4. Calibration curve of ERTA using proposed HPLC method.

Table. 3 Validation parameters for ERTA.	
Linearity range	5.00-120.00 µg/mL
Slope	0.2685
Intercept	0.2937
Correlation coefficient	0.9997
LOD	1.60 µg/mL
LOQ	4.85 µg/mL
Accuracy ± S. D	99.27 ± 0.41
Intraday precision	0.34
Interday precision	0.38

3.2.3. Accuracy and precision

The proposed method was found to be accurate, as % recovery ranged from 98.89 – 99.72. In addition, the method is precise, as the % RSD value is less than 2 in both intraday and interday precision. The obtained results of accuracy and precision are listed in (Table 4). The criteria accepted for recovery was % recovery near 100 % and for precision was % RSD not more than 2.

Table. 4 Accuracy, intra-day, and inter-day precision of the proposed method.

Concentration (µg/ml)	% R*	Intra-day precision % RSD*	Inter-day precision % RSD*
10	99.72	0.32	0.38
50	99.20	0.27	0.32
100	98.89	0.42	0.45

3.2.4. Specificity

The method proved its specificity as it was able to separate the drug from the degradant with sharp and uniform peaks as illustrated in (**Fig. 5**) and (**Fig. 6**).

3.2.5. Limit of detection and limit of quantification

Using the previously mentioned equations, LOD and LOQ were estimated. Detection and quantification limits results are presented in (Table 3). The method was able to detect and quantify ERTA in low concentration.

3.2.6. Robustness

The method is robust as % RSD under different conditions were less than 2. Obtained results are illustrated in (Table 5).

Table. 5 Robustness of ERTA using the proposed method.	
Temperature change (°C)	% RSD *
30±1	0.32-0.37
pH	% RSD *
4±0.1	0.15-0.27
Wavelength (nm)	% RSD *
224 ±1	0.27-0.39
Flow rate (mL/min)	% RSD *
1±0.10	0.23-0.38
*Average of 3 times	

3.3. Degradation behavior

ERTA was degraded under acidic, basic, and oxidative conditions resistant to photolytic and thermal degradation after being subjected to stress conditions. The acidic and basic chromatogram degradation revealed the appearance of new peaks of the degradation product. The degradant peak increases with time while the drug peak decreases with time. Chromatograms of acidic and basic degradation is shown in (**Fig. 5**) and (**Fig. 6**), respectively.

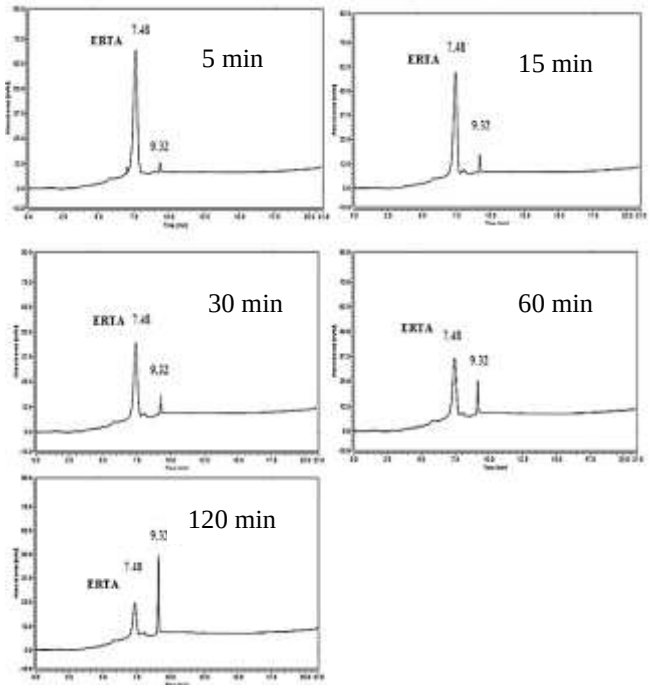

Figure 5. HPLC chromatogram of 100.00 µg/mL ERTA under 2N HCl under time interval 5, 15, 30, 60, 120 min.

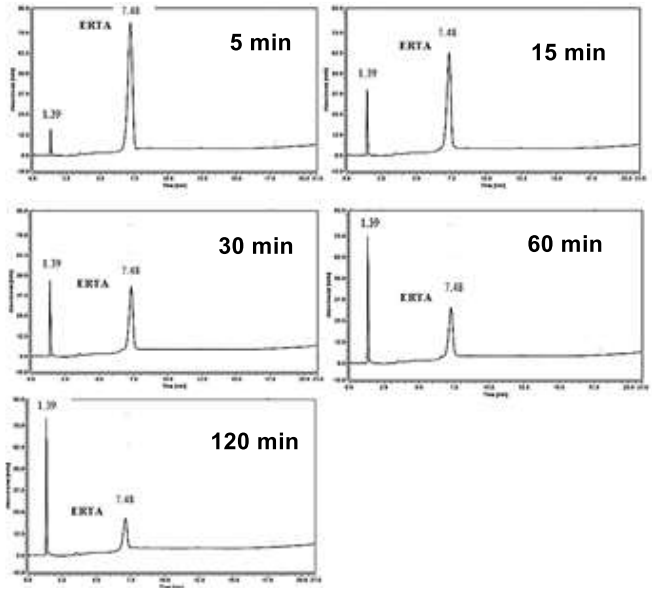

Figure 6. HPLC chromatogram of 100.00 µg/mL ERTA under 2N NaOH under time interval 5, 15, 30, 60, 120 min.

The drug most significant breakdown was seen during the oxidative process. This can be interpreted by the high suitability of the drug to oxidative conditions. Therefore, studying the kinetics parameters was not possible under oxidative conditions. Chromatogram of the drug under oxidative conditions is shown in (**Fig.7**).

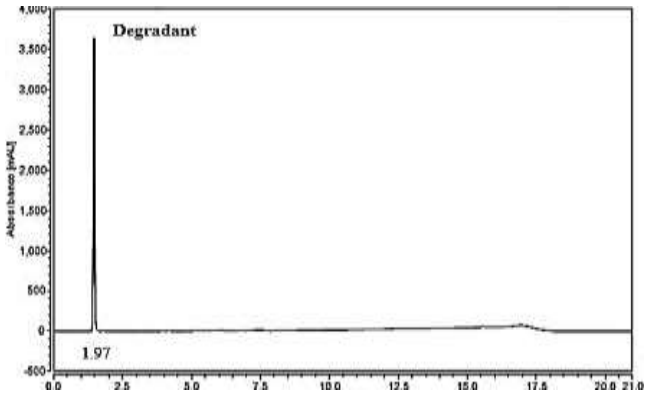

Figure 7. HPLC chromatogram of 100.00 µg/mL ERTA under oxidative condition after 5 minutes.

The drug showed stability under thermal and photolytic degradation as no additional peaks was observed in the chromatogram. A chromatogram of the drug under thermal and photolytic conditions is shown in (**Fig. 8).**

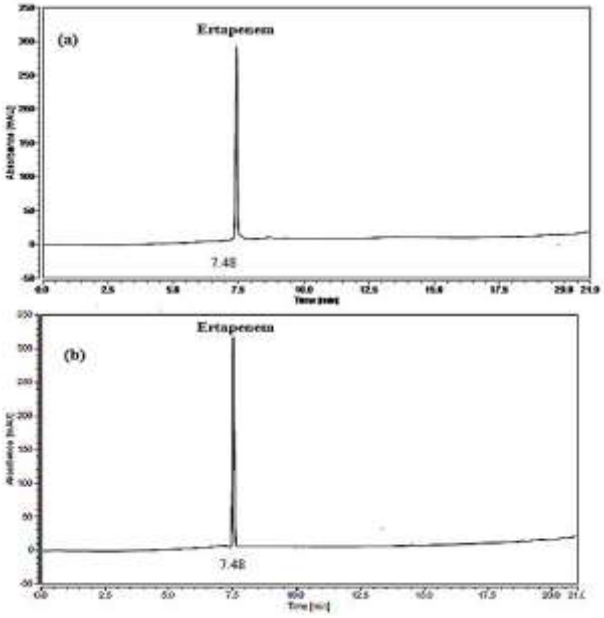

Figure 8. Chromatogram of 100.00 µg/mL ERTA under thermal stress conditions (a) and photolytic stress conditions (b).

Kinetics parameters were computed for acidic and basic conditions, while the oxidative was not estimated as the drug completely degraded after 5 min of exposure to hydrogen peroxide. The kinetics parameters included were k, t1/2 and t 90. The parameters were calculated after 120 min of exposure to acid and base. The parameters were calculated from the following equations:

Log (Ct) = log (C0) – Kt / 2.303

t ½ = 0.693/k

t90 = 0.105/k

where, Ct is drug concentration in µg/mL at time t, C0 is the initial drug concentration in µg/mL, k is the rate constant, t is the time in min, t ½ is the half-life of the reaction and t90 is the time that the drug takes to reach 90% of the initial concentration. Kinetics parameters calculation results are illustrated in (**Table. 6**). The results obtained showed that degradation rate under basic conditions is more than acidic one and there is a pseudo-linear relationship between time and 2+log Ct/C0 as shown in (**Fig. 9**).

Table. 6 Kinetics parameters under different stress conditions.

Kinetics parameter	Acidic hydrolysis	Basic hydrolysis
K per minute	0.0062	0.01101
$t_{1/2}$	111.13	62.94
t_{90}	16.83	9.54

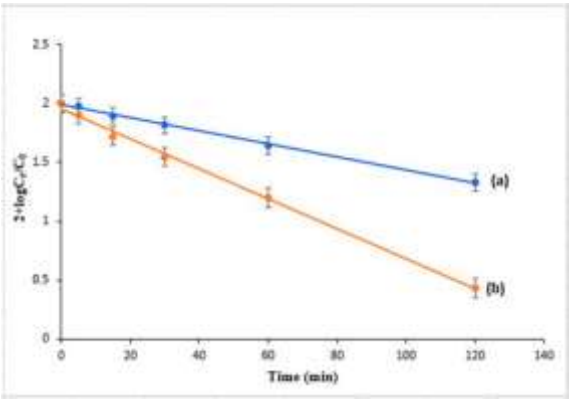

Figure 9. Pseudo-first order kinetics of 100.00 µg/mL ERTA in acidic conditions (a) and basic conditions (b).

3.4. MS characterization of ERTA and its degradation product

At first, the mass spectrum for ERTA was obtained as shown in (**Fig. 10**). After exposure of the drug to acidic, basic, and oxidative degradation, mass scanning of the degradation samples was performed after 2 h. The mass spectra under acidic, basic, and oxidative conditions are shown in (**Fig. 11**).

The structure of degradation products was expected based on the obtained mass data. It was identified by m/z values. The scanning range was 50-625 m/z. As reported in literature, penicillin, which contains a highly strained beta-lactamase ring, is susceptible to degradation under acidic, basic conditions due to amide bond breakage [37]. The same principle can be applied to ERTA antibiotic to expect the main degradation product to be obtained. It was found that the degradation product of ERTA is the open, four-membered beta-lactamase ring. Amid bond breakage in acidic conditions led to a molecular weight of 477.64, while in basic conditions it was 493.05. In oxidative conditions, the sulfur atom was oxidized which led to the extensive degradation of ERTA. The degradation product obtained under oxidative conditions was 507.87. The structure of the degradation products is illustrated in (**Fig. 12**).

3.5. Greenness assessment of the analytical method

The use and production of hazardous compounds should be decreased and eliminated as the main goal of green analytical chemistry. Hence, the method was evaluated by two greenness assessment tools, including analytical Eco-scale and AGREE tool.

3.5.1 Analytical Eco-scale

It is a useful, semi-quantitative tool that can be an alternative to green chemistry approaches [38]. It is a method used for assessing how environmentally friendly the analytical procedure is. It is based on deducing points that do not align with the green analysis. It is calculated as per the subsequent formula: analytical eco-scale score = 100- total penalty points. The outcome is rated on a scale. If the score > 75; excellent green analysis, > 50; accepted analysis, < 50; inadequate analysis [38]. Greenness assessment results using eco-scale are shown in (**Table. 7**). It was found that it is accepted green analytical method based on the score obtained.

3.5.2 AGREE tool and software

The analytical green calculator, which is based on green chemistry 12 principles, is a tool designed to help in assessing the environmental and occupational risks of certain analytical techniques. It can be used to compare analytical methods and choose the one that has the least effect on the environment [39]. The assessment findings are shown in a graph that includes overall score and measures how closely the technique adheres to each of the 12 principles of green analysis. Software results are shown in (**Fig. 13**). The score was obtained due to the following reasons: off-line analysis, minimum number of samples was used, off-line procedure, few steps for preparation of the sample, used device is automated, derivatization reagent was not used, low generation of waste, two analytes was determined during the run, low energy of used instrument, biobased reagents used, no toxic reagents and operator safety is high. Therefore, the method was evaluated as an environmentally green analytical method.

3.6 Proposed HPLC method comparison with reported method

The results of the proposed HPLC method were compared with the reported one as illustrated in **Table. 8**. The advantages of the method were as following; it has a wide linearity range, for the first-time kinetics parameters were calculated. In addition, mass spectrum for ERTA under acidic, basic, and oxidative conditions were reported and structure for degradation products were shown. The technique was assessed by analytical eco-scale and AGREE tool.

4. Conclusion

In the proposed study, a simple, sensitive stability indicating HPLC method was developed for ERTA determination as per ICH guidelines. The method proved to be accurate, precise, and selective. After applying different stress conditions to ERTA, the method was able to successfully separate the drug from the degradants. The degradation behavior of ERTA was studied and it was found that ERTA is stable under thermal and photolytic conditions and it was degraded under acid, basic and oxidative conditions. kinetics parameters were estimated. Moreover, degradation products characterization was carried out. Finally, the method was assessed by two greenness assessment tools, and it was found to be a green analytical procedure that can be used in further analysis in quality control labs.

Conflict of interest

The authors declare that they do not have conflict of interest.

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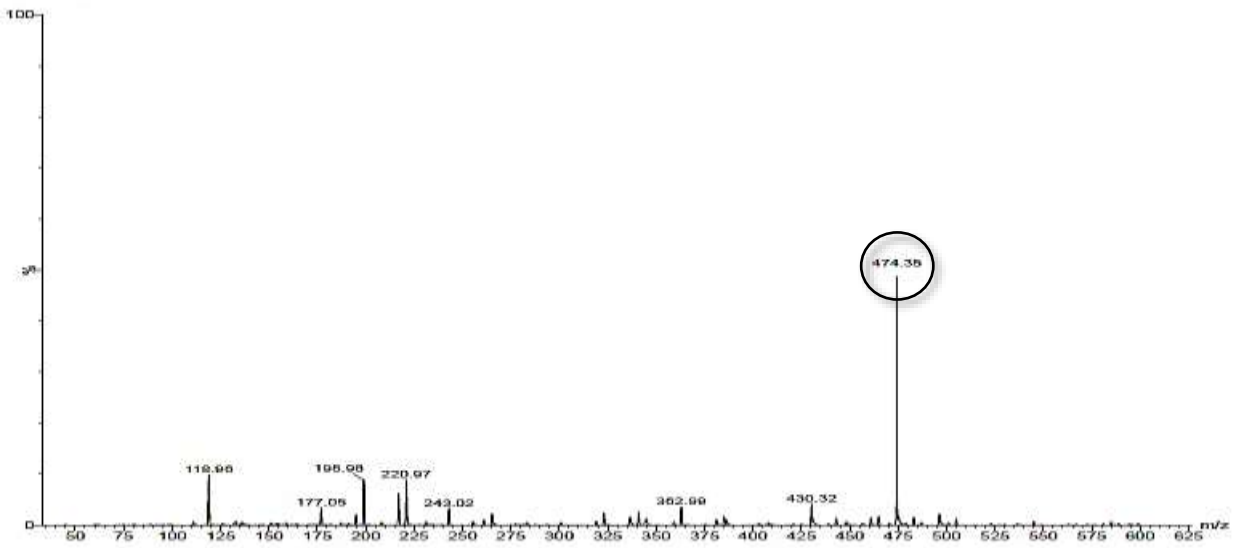

Figure 10. Mass spectrum of pure ERTA

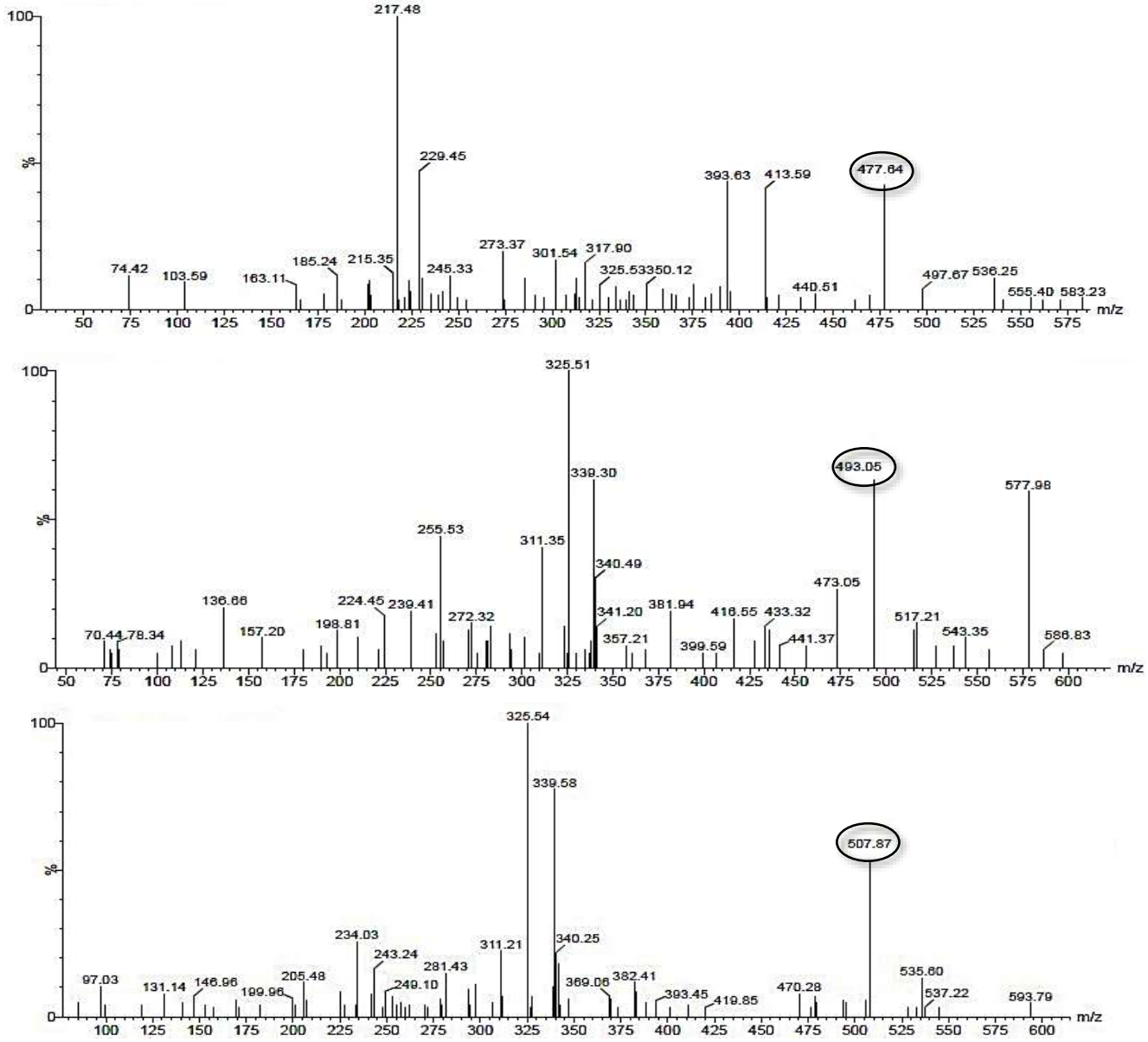

Figure. 11 Mass spectrum of acidic (a), basic (b), oxidative (c) degradation product of ERTA.

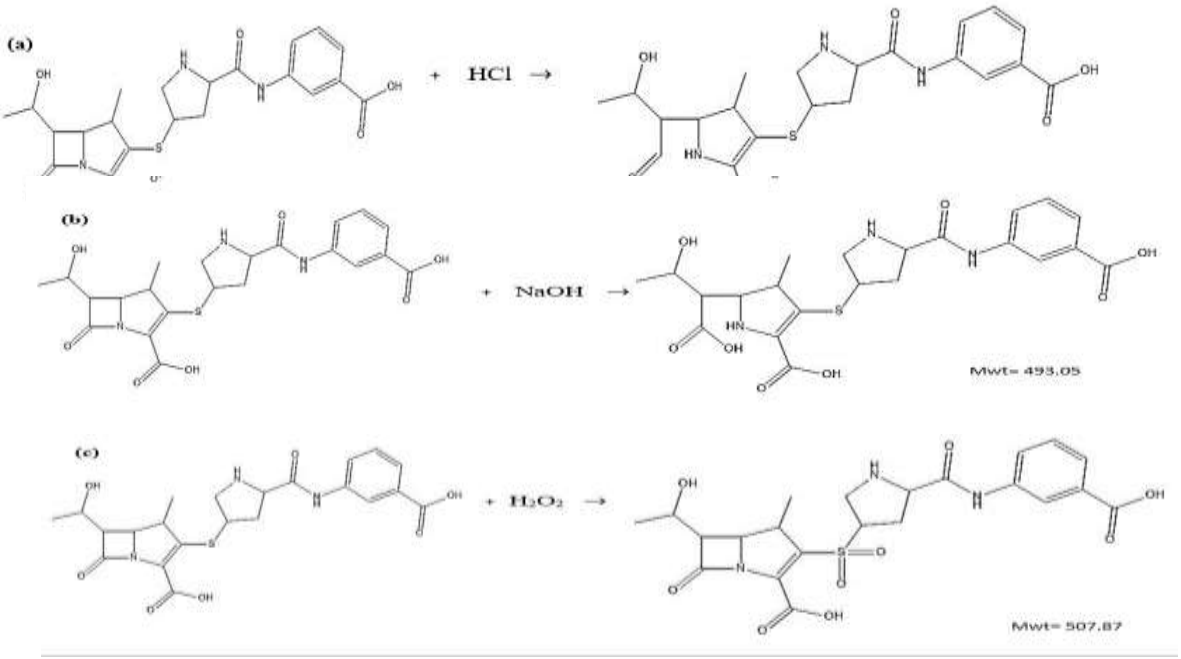

Figure 12. Proposed degradation pathway of ERTA in acidic (a), basic (b), oxidative (c). **conditions.**

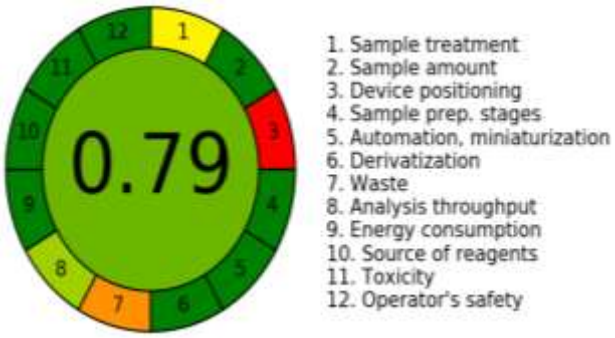

Figure. 13 Greenness assessment of the proposed analytical procedure by AGREE tool.

Table. 7 Analytical eco-scale penalty points

Type of reagent	**Penalty points**
Methanol	(More than 100 mL) = 18
Ortho phosphoric acid	Less than 10 mL = 2
Hazardousness	(None)= 0
Energy consumption	(Less than or equal to 1.5 kW h per sample) = 1
Waste production	>10 mL = 5
Total penalty points	26
Analytical Eco-Scale total score	74
Assessment	Acceptable green analysis

Table. 8 Comparison between the proposed method and reported method for ERTA stress testing.

Technique	Stress conditions tested	Kinetics parameters	Greenness assessment	Mass spectrum under stress conditions	Suggestion for Chemical structure of the degradant	References
HPLC-PDA	Acidic Hydrolysis Baic Hydrolysis Oxidative Hydrolysis Photolytic Hydrolysis Thermal Hydrolysis	K $t_{1/2}$ t_{90}	Analytical eco-scale and AGREE	Mass spectrum for acidic, basic, oxidative conditions was illustrated.	The structures of the degradation products were shown.	Proposed method
UV-Spectrophotometry	Oxidative Degradant Hydrolytic degradant	ND	Analytical eco-scale and NEMI	ND	ND	[22]
UV-spectrophotometry	Acidic Hydrolysis Baic Hydrolysis	ND	ND	ND	Suggested for acidic condition	[23]
HPLC-MS	Acidic Hydrolysis Baic Hydrolysis Oxidative Hydrolysis Photolytic Hydrolysis Thermal Hydrolysis	ND	ND	Illustrated	Suggested	[24]
HPLC-UV	Acidic Hydrolysis Baic Hydrolysis Oxidative Hydrolysis Photolytic Hydrolysis Thermal Hydrolysis	ND	ND	ND	ND	[25]
HPLC-UV	Thermal Hydrolysis	ND	ND	ND	ND	[26]
HPLC-MS	Acidic Hydrolysis	ND	ND	Illustrated under acidic condition	Suggested for acidic condition	[27]
HPLC-UV	Acid base catalysis under effect of water	Kinetics of degradation in aqueous solution was studied	ND	ND	ND	[28]

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